An analysis of establishing the natural order of water at interfaces with Biological Systems

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Water interacts with various biomolecular surfaces in a complex and often counterintuitive manner. The analysis of these molecular interactions and the relationship that evolves in the system is of a phenomenon that is not greatly understood. Here I will discuss the process-property-function that is associated when biological structures are placed in models for observation with water at the molecular and microscopic levels. Case studies will be presented to explain the dynamic nature of water and its nebulous tendency to conform and behave differently when presented with a different biological interface. This perspective will give us an insight into understanding this often ignored yet critical piece of the thermodynamic functionality water has with biological structures. I will also introduce cutting edge research using the knowledge gained at the pico and nano levels and applying these physical and biological concepts. This review will discuss the basic interpretations of these concepts and the path forward to gain a deeper understanding of the biological interaction with water.

Water as we understand is capable of doing a great deal of benefit for Biological systems. The mechanism for interaction is understood using polarity and arrangements of molecular layers surrounding the interface and their energy potential as they form when confronted with an interface.[5] The nature of water plays a role in many different biological processes from protein folding, enzyme kinetics to hydration and ATP production.

A. Properties and Observations

Water is unique in liquid phase because of its ability to form a network in 3dimensional space network of hydrogen bonded molecules. This is a consequence of the four fractional charges which when directed in three dimensional space form a tetrahedron.[2] The weak bonds with a relatively low force of Hydrogen bonding resulting from the dipole moment from the lone pair of electrons on the Oxygen negatively interacting with the positive pole of the neighboring molecule's Hydrogen allow for waters' ability to layer as a liquid. However, when water becomes ice, the bonding structure becomes rigid as these bent interactions form straight lines and form crystalline structures with a strong network of inline bonding.[2,3] Water is an excellent solvent when thinking about biological interactions as it contains a great ability to dissolve a variety of solutes for charged and polar solutes. When confronted with hydrocarbons, which will

be discussed in great importance, does not easily dissolve these molecules.[2]

I. Energy Consequences

As water plays an important part in the growth and development of biological systems, its method of assimilation and usefulness as a source of energy is extremely important at the molecular level. As water molecules form around hydrophobic regions a layering effect occurs as negative pressures build around regions where water cavitation is prevented. A study done at Columbia University experimenting with spores have shown spores to exhibit extreme characteristics when confronted with these similar circumstances. By manipulating the hydration level of the spore, it was able to be maneuvered in a fashion that its response generated an electric current and thus energy was able to be harvested.[6] This will be reflected upon later.

II. Layering

As water comes into contact with surfaces a pattern emerges as to its density. When using atomic force microscopy, when the tip has been close to the solid surface of a solid and liquid interface, AFM measures the oscillations of a period that is equal to the liquid molecules diameter.[7] Kimura et al. used this method to further develop this image of a solvation layer via intermediate structures between layers cross-sectional in a vertical direction. Using the proton pump bacteriorhodopsin (bR) of *Halobacterium salinarum*, the study investigated 2D hydration with the membrane. A fairly often studied monomer, the transmembrane protein established hydration structures at the interface with solution. The intervals between layers become larger the farther the distance from the interface, giving a strong indication that the interaction forces are weakest with the water molecules at this outer layer.[4]

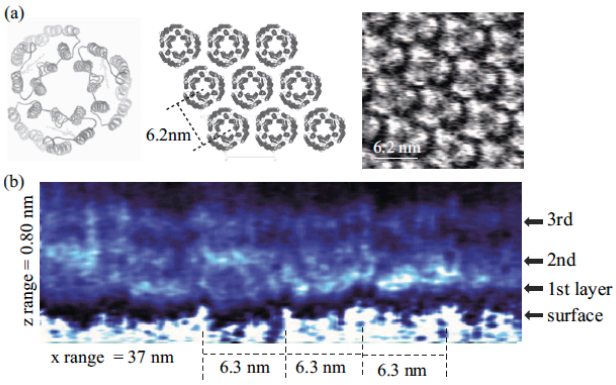

Figure 1 (Kimura et al., JCS) (a) The images under a shows bR in its quaternary structure at the left. It is when a bR molecules form 7 transmembrane alpha helixes line up forming a tertiary structure. The second image shows the quaternary structure bR forms as a trimer of 3 tertiary structures forming a hexagon formation. The far right image is the 2D hexagonal structure imaged with FM-AFM on its cytoplasmic side. (b) Represents the 2d hydration model measured through FM-AFM. It can clearly be seen as that three structured water molecule layers form around the interface. The intervals become larger as distance from the interface increases up to the top layer reflecting that the interaction forces are its weakest with the water molecules of this region.[4]

Water also exhibits this layering effect and the force of interaction is quantifiable when measured with a lipid raft surface.[8] Water existing above the layers is referred to as bulk water as it has no discernable configuration and high in entropy.[11]

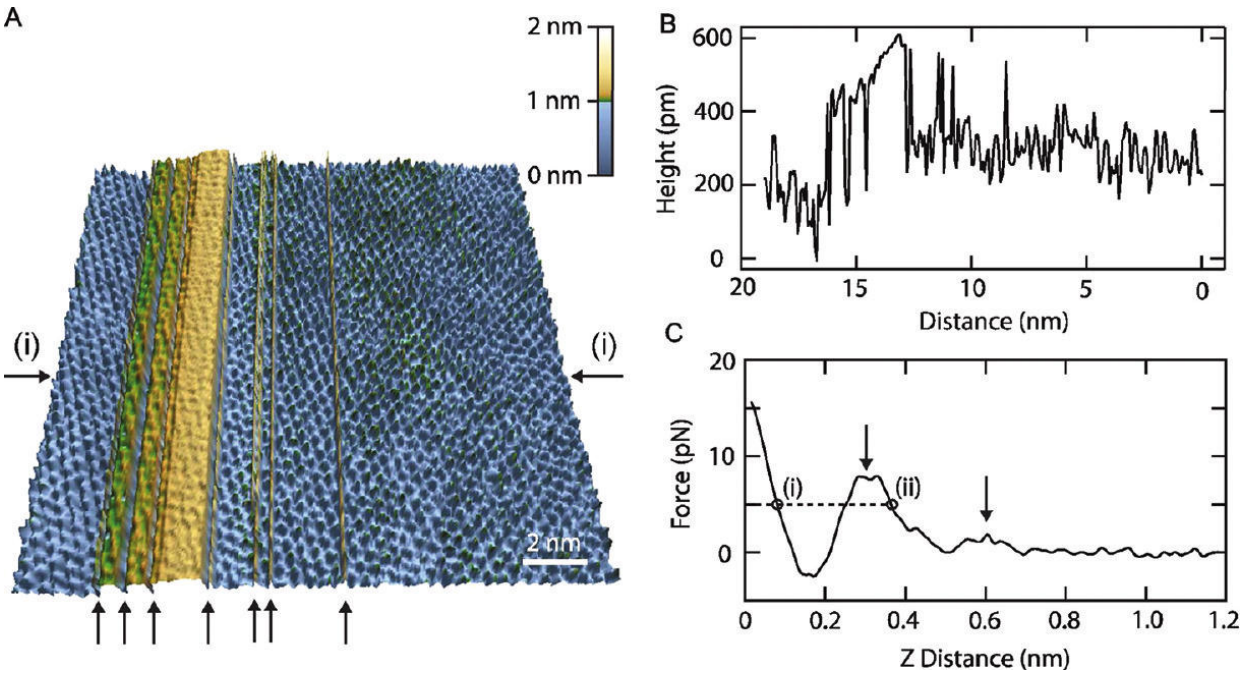

Figure 2. (Sheikh and Jarvis, JACS) (a) A lipid raft system was constructed of varying height was constructed and measured hydration layering absorbing to the interface. The arrow point to regions where the AFM tip jumps between the surface and the hydration layer about it. (b) represents the cross section of image along the path (i). (c) Representation of the measured force from the AFM tip and surface as a function of the tips distance above the surface, with arrows reflecting oscillation peaks which are associated with AFM tip transitioning through hydration layers.[8]

III. Adhesion effects

This phenomenon can best be explained when there is an interface that results in water behaving in a manner different from hydration layering. When faced with water at various pH levels, mussel foot proteins which are natural adhesives and amyloid were combined to create nanofibers that were robust underwater and reached adhesion energies 1.5 times greater than any bioengineered protein based adhesive found to date.[9] This implication that despite the interfacial force water exerts, proteins are very capable of overcoming and assembling polymers for greater adhesion.

The adhesion a protein undergoes as a result of its natural tendency to fold is regulated as its hydrophobic collapse involves the movement of water as amino acids are forming pairs.[10] The water molecules that are in the interface hydration "shell" are of critical importance as these proteins bend. The residence time a molecule can can sit in the hydration layer as the neighboring protein surface may result in partial desolvation with the amino acid as its residence may be shorter lived as the protein makes its connections. This will be mediated, of course, by the dynamics of the layer.[1]

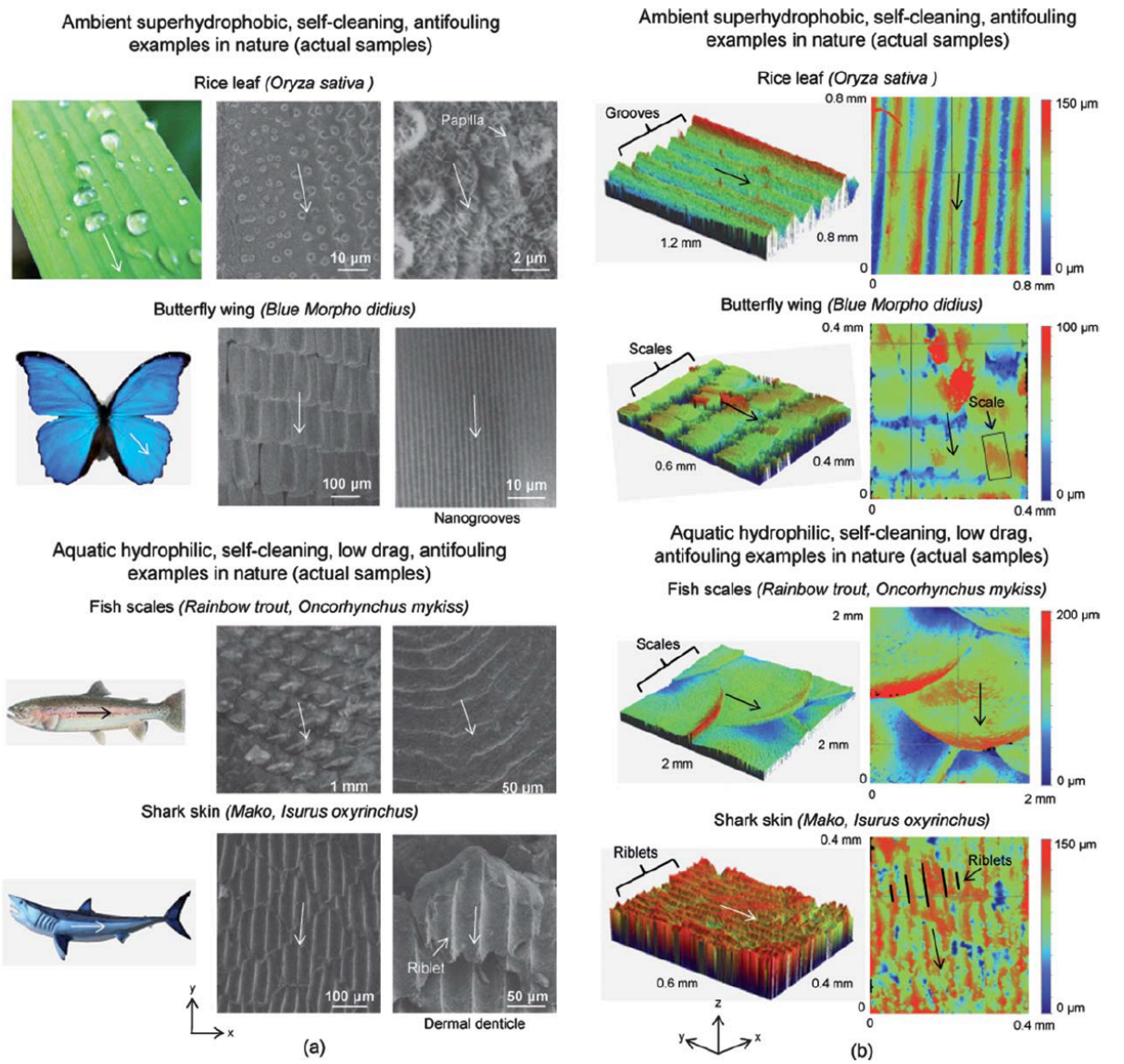

Figure 3. (Bixley and Bhusian, RCS) Interfaces found naturally occurring in the environment. (a) Comparison between SEM of super hydrophobic interface exhibiting low adhesion vs hydrophilic interfaces which exhibit high adhesion. (b) Map of heights done with an optical profiler. Arrows show direction of travel of water droplet.[12]

As shown in figure 3, water can coalesce when interacting with a super hydrophobic surface that is conducive to forcing the water droplet away from it as the lotus effect. This "self-cleaning" property of directional moving water is also found in hydrophilic examples. However, on interfaces of low adhesion where the force to bead the water mimics the the low drag hydrophilic nature of shark skin and fish scales, give the rice leaf and butterfly wing a distinct effect. The wetting effect of the aquatic animals were able to give the water molecule a flattened shape and drastically reduce the drag. Whereas the rice and butterfly contain super hydrophobic grooves that bead and channel the water molecule.[12] This type of behavior

is exhibited in spider silk as the water beads and coalesce at spindle knots in the web.[13]

2. Role in Biological Processes-Enzyme Activity Implication and Free Energy of the system.

Water is integral to the biological process of enzyme activity. Water molecules are the solute for which most co-enzymes interact with vitamins. The breakdown and integration of vitamins are dependent on abundance of polar water to interact with the vitamin and a co-enzyme is formed as a result to synthesize the vitamin. A deficiency of water will inhibit this pathway and result in a myriad of health issues.[14] The ability for water to molecularly act both as a proton donor or acceptor helps in this process of catalysis. Carbonic Anhydrase has showed in experiments that water as a solvent in interactions has increased enzyme specificity and versatility in enzyme-substrate interactions.[18]

When discussing layering earlier it can be said that first hydration layer, the layer closest to the interface forms a type of shell. This relatively high amount of water molecules can be described as high density water, as there are more molecules that surround the interface as the interaction forces are greatest in this region. The farther away from the interface the less compacted the molecules, the lower the density of molecules. This is important to consider when thinking about how a solute has a particular effect on the enzyme behavior and its kinetics. The free energy of a system is manipulated when a radical is placed into it. For instance, if you add a salt particle to the water, it will affect the conformations of water molecules and ultimately change the characteristics of the water and how it adjusts to its surfaces. This can be seen in difference of boiling and freezing points.[15]

An experiment done by Beauchamp and Khajehpour highlights the interactions water has with enzymes and when free particles are added, changes the way water acts to stabilize or de-stabilize a protein. For this experiment, a number of observations were made about solvation layer of water as they used different salts to form high density layers around the salt as which allowed for the promotion and formation of low density water molecules to surround the protein hydration layer. This enhanced hydrophobic interaction caused a compact, rigid and less active form of the RNase t1 to be stabilized. It was observed that this loss of enzyme activity was further degraded as the salt concentration increased. The salt molecule if replacing a single water molecule in the low density water of the protein hydration layer will affect all the surrounding water molecules in the given area, thus dramatically reducing the hydrogen bonding of the molecules and thermodynamically agitating the protein. The different the salt species species, the different the effect on the K value. For all observed instances of species, the Michaelis-Menten graph was effected. Ignoring the effects of NH_4Cl as it has an ability to Hydrogen bond, it was observed:

$$\left(\frac{K}{1+K_0}\right)_{Mg^{2+}} > \left(\frac{K}{1+K_0}\right)_{Ca^{2+}} > \left(\frac{K}{1+K_0}\right)_{Li^{+}} > \left(\frac{K}{1+K_0}\right)_{Na^{+}} \approx \left(\frac{K}{1+K_0}\right)_{K^{+}}$$

Which indicates that the the salts may very well affect the enzyme by shifting the molecular alignments of the water molecules.[16]

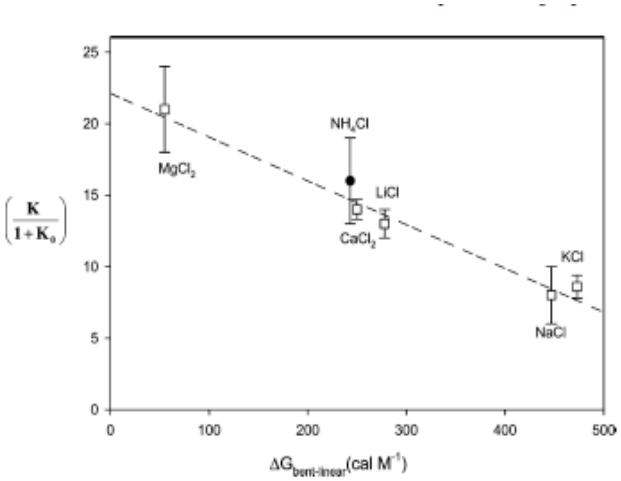

Figure 4. (Beauchamp and Khajehpour, ACS) The free energy difference (ΔG^0) between High Density and Low Density Water of various salt solutions plotted against dependence of the $K/(1+K_0)$ parameter of the RNase t1 protein.

The ability for salt beyond the water molecules first solvation shell and stabilize low density water in farther out layers prevents the solubilizing of nonpolar entities as solvation depends on the ability for low density water molecules to readily become high density thus when salts are dissolved in water, thus the ordering of water molecules exhibits a downward linear trend in free energy vs activity.[16]

In the understanding of how water can affect free energy of the system, it is critical to understand the importance that pure water has the ability to arrange and rearrange its molecules for the benefit of the system. To refer back to the experiments conducted with spores, water can turn these small bacterial spores into actuators and quantify energy from the mutual interaction. In a follow on experiment with spores, through the natural process of evaporation, Chen et al. were able to ramp up a nanoscale energy conversion between water particles in humidity and spore into a device that could exploit this nanoscale phenomena maximizing surface to volume ratios. They were able to take this relatively small and often neglected property and turn it into an inexpensive means to produce usable power.[17]

Conclusion

The importance of water to biological systems is extremely delicate. As seen, a single salt molecule can offset its ability to facilitate processes. At the nanoscale level, it is important to understand the behavior of water, and how it affects the surroundings. The interface between water and biological systems is an up and coming research field that has limitless potential for growth and understanding. After all, life was born out of water and evolution occurred partly because of its abundance. Energy is derived from water at the nano level all the way up to the mega level with hydroelectric dams. Finding new ways to derive energy from water is important to getting ourselves away from fossil fuels. The best way to understand this, is to start small, where water meets biology.

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